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Invention: METHOD AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS

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10/677,956

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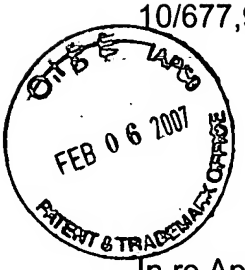

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Group Art Unit: 1648
ZEBEDEE et al.	Examining Attorney: Zachariah Lucas
Serial No.: 10/677,956	
Filed: October 1, 2003	Date: February 6, 2007 Pasadena, California
For: METHODS AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS	

SUBMISSION OF MARKED UP PAGES OF WANG UNITED STATES PATENT NO. 5,106,726 FILE HISTORY

Commissioner for Patents
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Alexandria, VA 22313-1450

The attached 24 pages are from the Wang Patent file history and are the same 24 pages referred to in the Supplemental Amendment filed January 31, 2007 at page 18, second paragraph. These enclosures may have been omitted in the copy of the file. Any omission is regretted.

Date: February 6, 2007

Respectfully submitted,

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According to the present invention, a peptide composition useful for the detection of antibodies to HCV and diagnosis of NANBH comprises a peptide selected from the group of peptides with the following sequences:

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

wherein X is -OH or $-NH_2$, and analogues, segments, mixtures, combinations, conjugates and polymers thereof.

The amino acids in this application are abbreviated as shown herein below:

A= Ala= alanine,

R= Arg= arginine,

D= Asp= Aspartic acid,

N= Asn= asparagine,

Q= Gln= glutamine,

E= Glu= glutamic acid,

L= Leu= leucine,

K= Lys= lysine,

H= His= histidine,

T= Thr= threonine,

G= Gly= glycine,

I= Ile= isoleucine,

F= Phe= phenylalanine,

S= Ser= serine,

W= Trp= tryptophan,

Y= Tyr= tyrosine,

V= Val= valine,

C= Cys= cysteine,

P= Pro= proline

An example of a combination is: Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X wherein X is -OH or $-NH_2$. An example of a segment of Peptide II is: Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X wherein X is -OH or $-NH_2$ (IIF). An example of a segment of Peptide III is:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X wherein X is -OH or $-NH_2$ (IIID). An example of a segment of Peptide IX is Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IXC).

The present invention also includes a highly sensitive and accurate method of detecting antibodies to HCV in body fluids and of diagnosing HANBH comprises the following steps:

A. Preparing a peptide composition comprising a peptide selected from the group having the following amino acid sequences:

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

(4)

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIM)

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

wherein X is -OH or $-NH_2$, and analogues, segments, mixtures, combinations, conjugates and polymers thereof; and

B. Using an effective amount of the peptide composition as the antigen in an immunoassay procedure.

Further, according to the present invention, the peptides by themselves, or when coupled to a protein or a polymeric carrier of homo or hetero dimers or higher oligomers

(13-5 and 13-6). The results were screen tested in a blood bank setting.

Figure 14-1 provides a study of serum samples collected over a ten year period of time from a NANBH patient who sero-converted after receiving HCV infected blood. The samples were tested by a third EIA format designated as C (coated with Peptides IIH, V, and VIIE at 5, 3 and 2 ug/mL respectively) in comparison to two other EIA formats (designated as A and B.)

Figure 14-2 provides another kinetic study with serum samples, kindly provided by Dr. D. Bradley of Center for Diseases Control, from a chimpanzee which sero-converted after being inoculated with a well-characterized strain of HCV and contracted NANBH. These samples were tested by the HCV EIA Format C, in comparison to a RIA using rDNA based HCV C-100 protein as the antigen. The ALT levels are also indicated with each bleed as a reference parameter.

Figures 15-1 and 15-2 both provide a side-by side data comparison via x-y plots with samples from hemodialysis patients, kindly provided by investigators at the Japanese National Institute of Health. The results were obtained by using the peptide based HCV EIA Format C (coated with peptides derived from both the structural and non-structural proteins containing IIH, V and VIIIE at 5, 3, and 2 ug/mL respectively), HCV EIA Format A (coated with peptides derived from the nonstructural protein region containing IIH and V at 5 and 3ug/mL respectively), and the recombinant HCV C-100 protein based EIA.

by stimulating the production of antibodies to HCV. These peptides are arranged in the following sequences:

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

wherein X is -OH or $-NH_2$.

In selecting regions of the HCV protein for epitope analysis, peptides in the 40mer size range with amino acid sequences covering the complete HCV C-100 protein and the postulated core protein were synthesized. These were tested for their immunoreactivity with serum from a patient positively diagnosed with HCV infection. Six overlapping peptides from the HCV C-100 protein region designated as I, II, III, IV, V and VI and two adjacent peptides form the postulated core protein region designated as VIII and IX were identified to have specific immunoreactivity with the positive HCV serum. Another peptide VII and its fragments, C-terminal to this immunodominant region, was also found to have moderate immunoreactivity with a sub population of HCV positive sera. See Example 12. Peptide IIH, another analogue of Peptide II, with five additional amino acids to the N-terminus has been found to be highly immunogenic and contains an additional epitope recognizable by antibodies in sera from patients with acute phase NANBHV infection (with elevated ALT levels). The amino acid sequences of the peptides are as follows:

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(9)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Glu-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

The six peptides I, II, III, IV, V and VI span a region of 90 amino acids:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Arg-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe

and were found to have specific immunoreactivity with the positive control serum. Table 1 shows the amino acid sequence of this immunodominant region of the HCV protein, and presents the amino acid sequence of the six chemically synthesized peptides, designated as I to VI and segments (A to H) thereof.

Another two peptides (VIII and IX) spanning a region of 119 amino acids located inside the 5' terminal of the postulated HCV core protein:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

were found to have specific immunoreactivity with a representative panel of well-characterized HCV antibody positive sera.

Table 7 shows the amino acid sequence of this immunodominant region of the postulated HCV core protein, and presents the amino acid sequence of the ten chemically synthesized peptides. They were designated, as Peptides VIII and IX with segments (A to D) thereof. Each of these peptides was coated at 5ug/mL in a 10mM sodium bicarbonate buffer (pH 9.6) onto polystyrene microwell plates and tested in a three step 45 minute enzyme immunoassay.

Table [illegible]

CHARACTERIZATION OF THE IMMUNODOMINANT REGION OF THE HCV 5CO-C100 FUSION POLYPEPTIDE:

	[illegible].VLSS.NPAII.PORE[illegible].REF.DEMEE.CSQHL.PYIEQ.GMMLA.EOFKO.KALGL.LOTAS.ROAEV.IAPAV.OTNWO.KLETF.WAKHM.WNF	RELATIVE (%) IMMUNOREACTIVITY
IA	GL.LOTAS.ROAEV.IAP	3.0
IB	KO.KALGL.LOTAS.ROAEV.IAP	18.1
IC	MLA.EOFKO.KALGL.LOTAS.ROAEV.IAP	23.[illegible]
ID	EO.GMMLA.EOFKO.KALGL.LOTAS.ROAEV.IAP	24.8
IE	ML.PYIEO.GMMLA.EOFKO.KALGL.LOTAS.ROAEV.IAP	38.2
IF	SS.CSQHL.PYIEO.GMMLA.EOFKO.KALGL.LOTAS.ROAEV.IAP	45.5
IIA	GMMLA.EOFKO.KALGL	3.1
IIB	PYIEO.GMMLA.EOFKO.KALGL	24.3
IIC	CSQHL.PYIEO.GMMLA.EOFKO.KALGL	41.7
IID	DEMEE.CSQHL.PYIEO.GMMLA.EOFKO.KALGL	44.9
IIE	[illegible]REF.DEMEE.CSQHL.PYIEO.GMMLA.EOFKO.KALGL	57
IIF	PREE[illegible].[illegible]REF.DEMEE.CSQHL.PYIEO.GMMLA.EOFKO.KALGL	99
IIG	[illegible].PORE[illegible].[illegible]REF.DEMEE.CSQHL.PYIEO.GMMLA.EOFKO.KALGL	92.2
IIH	SS.NPAII.POREV.LYREF.DEMEE.CSQHL.PYIEO.GMMLA.EOFKO.KALGL	101
IIIA	EF.DEMEE.CSQHL.PYI	4.9
IIIB	[illegible].LYREF.DEMEE.CSQHL.PYI	26.2
IIIC	[illegible].POREV.LYREF.DEMEE.CSQHL.PYI	85
IIID	SS.NPAII.POREV.LYREF.DEMEE.CSQHL.PYI	100
IIIE	SR.VLSS.NPAII.POREV.LYREF.DEMEE.CSQHL.PYI	99
IIIF	[illegible].VLSS.NPAII.POREV.LYREF.DEMEE.CSQHL.PYI	95
IV	SS.NPAII.POREV.LYREF.DEMEE.CSQHL.PYIEO.GMMLA.EOF	93.8
VIA	AS.ROAEV.IAPAV.OTNWO.KLET	3.4
VIB	GL.LOTAS.ROAEV.IAPAV.OTNWO.KLET	43.6
VIC	KO.KALGL.LOTAS.ROAEV.IAPAV.OTNWO.KLET	44.8
VID	LA.EOFKO.KALGL.LOTAS.ROAEV.IAPAV.OTNWO.KLET	46.0
VIE	EO.GMMLA.EOFKO.KALGL.LOTAS.ROAEV.IAPAV.OTNWO.KLET	54.[illegible]
VA	AV.OTNWO.KLETF.WAKHM.WNF	1.3
VB	EV.IAPAV.OTNWO.KLETF.WAKHM.WNF	17.8
VD	AS.ROAEV.IAPAV.OTNWO.KLETF.WAKHM.WNF	23.[illegible]
VC	GL.LOTAS.ROAEV.IAPAV.OTNWO.KLETF.WAKHM.WNF	32.5
V	KO.KALGL.LOTAS.ROAEV.IAPAV.OTNWO.KLETF.WAKHM.WNF	93.9

The underlined amino acid residues exert (—) marginal, (—) moderate, or (___) strong immunoreactivity

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X (VIIIE)

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IXD)

Peptides XIIIE and IXD were also found to have the highest reactivity in this region.

Assays for antibodies to HCV based upon chemically synthesized peptides show several advantages over assays utilizing biologic based immunoadsorbents. The peptides can easily be synthesized in gram quantities by using automated solid-phase methods, thus providing a reproducible antigen of high integrity with consistent yields. The presence of other antigens from biological systems precludes such reproducibility. More importantly, non-specific reactivities seen in uninfected individuals are likely to be due to the heterogeneity of the preparations used for assay. This is particularly true for assays using biologically based immunoadsorbents. In these processes, the host antigens are frequently co-purified with the desired viral protein(s). Antibodies to these contaminating antigens are frequently found in normal individuals, thus resulting in false-positive results.

The assay of the present invention clearly minimizes such false-positive reactions as encountered in the other assay systems and, at the same time, shows a high sensitivity to truly positive sera by the substantially increased signal-to-noise ratio. This increased signal-to-noise ratio probably resulted from the purity of the immunoadsorbent. The

assay of the present invention is also highly specific, in that the mean S/C ratios for HCV carriers are about 80-200 times the mean S/C of those of the non-infected individuals. For a representative example, see Figs. 3-1 and 3-2.

The peptides useful as solid phase immunoadsorbents for the detection of antibodies to HCV were synthesized by the "classical" Merrifield method of solid phase peptide synthesis using side chain protected t-Boc-amino acids to correspond to the following amino acid sequences:

(17)

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

wherein X is $-NH_2$.

Other analogues, segments and combinations of these peptides may be prepared by varying the amino acid sequences either by adding, subtracting, substituting, or deleting desired t-Boc-amino acid(s).

Following completion of assembly of the desired blocked peptide on the resin, the peptide-resin is treated with anhydrous hydrofluoric acid to cleave the peptide from the resin. Functional groups of amino acids which are blocked during synthesis by benzyl-derived blocking groups are also cleaved from the peptide simultaneously. The free peptide is then analyzed and purified by high performance liquid chromatography (HPLC) and characterized biochemically by amino acid analysis.

Longer peptides with more than about 50 amino acids may also be prepared conveniently using well known recombinant methods. The known nucleic acids codons for each of the amino acids in the peptide may be utilized and synthetic genes encoding such peptides constructed. The synthetic gene may be inserted into vector constructs by known techniques, cloned and transfected into host cells, such as E. coli, or yeast. The secreted polypeptide may then be processed and purified according to known procedures. The peptides synthesized

with Peptide IC increases significantly, followed by a marginal increase with Peptide ID, and additional increases with Peptides IE and IF. This indicates that in the HCV Peptide I series, two clusters of amino acid residues, namely LAEQF and HLPYI, are contributing to the antigenic determinant(s) of the HCV Peptide I. Similarly, a cluster of residues namely EKSSQHLPYI is contributing to the immunoreactivity of the HCV Peptide II series; another cluster of residues namely EGKPAIIIDR is contributing to the immunoreactivity of HCV Peptide III series and two clusters of residues, namely TILVT and EVIAR are contributing to the immunoreactivity by HCV peptides IV and V series. As shown on the bottom of Fig. 1-1, a total of six spaced clusters of amino acid residues representing discontinuous epitopes in this immunodominant region of the HCV protein are identified as contributing to the specific HCV immunoreactivity with serum sample 1.

Figure 1-2 illustrates an immunoreactivity profile for serum sample 2 when tested on a total of 31 overlapping peptides in the HCV Peptide I, II, III, IV, V and VI series. There is a clear difference between the immunoreactivity profiles of serum samples 1 and 2. The immunodominant epitope, as marked by residues EGKPA and IIPDREV, is located towards the N-terminus of the region.

Figure 1-3 illustrates an immunoreactivity profile for serum 3 when tested on the same 31 HCV peptide panel. Through this extensive epitope mapping analysis, serum sample 3 was found to have a similar immunoreactivity profile to that of serum sample 2.

(f) individuals with elevated (>100 I.U./L) alanine aminotransferase (ALT) enzyme activity, (n=174); (on both IIG and IIF/IIID plates)

(g) individuals positive for antibodies to retroviruses HIV-1(n=100), HIV-2(n=10), HTLV-I/II(n=14); all asymptomatic, (total n=124); (on both IIG and IIF/IIID plates)

(h) individuals with AIDS, ARC(n=200) or ATL (n=170) disease, (total n=370); (on both IIG and IIF/IIID plates) and

(i) individuals with autoimmune disease (n=20). (on IIG plates only)

(j) recombinant SOD/HCV C-100 HCV-EIA repeatably reactive specimens obtained from a random donor population, (n=23). (on both IIG and IIF/IIID plates).

Results obtained from groups (a) and (b) are presented in Figs. 2-1 and 2-2 respectively (data obtained on IIG plates only), from group (c) in Figs. 3-1 and 3-2; from groups (d) to (i) in Fig. 4, from group (j) in Table 3 and Figs. 5 and 6.

In brief, as shown in Figs. 2-1 and 2-2, a comparison, by signal to cutoff ratio, between the peptide based HCV-EIA of the present invention employing peptide IIG and that of recombinant SOD/HCV C-100 protein based HCV-EIA produced by Chiron/Ortho. Similar dilution titers and equal ability to identify date of sero-conversion, the two parameters indicative of each assay's sensitivity, are obtained for both assays. However, the assay according to the present invention is more sensitive and confers a higher signal to cutoff ratio to its positive specimens.

center of the wells. In this experiment, a P/C ratio of 20 was set as the assay cutoff value, i.e. a positive agglutination pattern had a ratio of <20 and a negative pattern, >20.

(41)

A total of 20 rDNA HCV EIA repeatably reactive specimens were tested for antibodies to HCV in the above-described HCV passive hemagglutination assay (PHA) employing Peptide IIG-BSA conjugate as the solid phase. Figure 6 provides a correlation study between the peptide based HCV PHA and the recombinant based HCV EIA by their respective P/C and s/c ratios. All samples with s/c EIA ratios higher than 3 were found to be positive with the HCV PHA test. With the exception of one, all specimens having borderline s/c ratios (between 0.9 to 2) scored as negative in this PHA test.

EXAMPLE 4

Detection of Antibodies to HCV By An Agglutination Assay Utilizing As the Solid Phase Immunosorbent Gelatin Particles, Erythrocytes Of Different Animal Species, Or Latex Particles Coated With a Mixture of HCV Peptides

One mL thoroughly washed erythrocytes, gelatin particles, or polystyrene latex particles are coated with the HCV peptide mixture, or conjugates thereof at an effective concentration. The peptide mixture, or conjugates thereof, coated cells or particles are then incubated with serially diluted serum samples in the wells of a 96-well U-shaped microplate or on a slide. After being left at room temperature for about an hour, or a few minutes in the case of latex particle based microagglutination, the settled agglutination pattern on the bottom of each well or on the slide is read; and the highest dilution showing a positive reaction is recorded.

EXAMPLE 15

Detection of Antibodies To HCV By Peptide Based Enzyme-Linked Immunosorbent Assay Using Format C, Format D, Format A

The following four groups of specimens:

(a) individuals with AIDS, ARC(n=63);

(b) individuals positive for HBsAg, (n=50);

(c) individuals positive for antibodies to HBc protein, (n=22); and

(d) individuals with elevated (>100 i.u./L) alanine aminotransferase (ALT) enzyme activity, (n=86).

were analyzed on representative HCV peptide based EIAs according to the present invention, with the plates coated either with (i) peptides IIH and V at 5 and 3 ug/mL each (Format A), (ii) peptides IIH, V and VIIIE at 5, 3 and 2 ug/mL each (Format C, containing both the HCV core and nonstructural peptides) or (iii) Peptides VIIIE and IXD at 2 and 2 ug/mL each (Format D, HCV core peptides only).

Results obtained from the screening of a total of 221 well-characterized clinical specimens previously categorized into four groups, from (a) to (d) using a representative lot of peptide coated plates EIAs formatted as A, C or D were plotted on histograms as shown in Figs. 12-1, 12-2 and 12-3.

Out of a total of 63 AIDS/ARC patient samples analyzed, 46.0%, 55.6% and 50.8% of the patients were found to be HCV antibodies positive using EIA formats A, C and D respectively. Out of 50 HBsAg positive individuals, 36.0%, 42.0% and 36% of the individuals were found to also be HCV antibodies positive using EIA formats A, C and D respectively. Out of 22 HBc antibody positive individuals, 27.3%, 22.7%, and

18.2% were found to be HCV antibodies positive as detected by EIA formats A, C and D. Out of 86 patients with an elevated ALT levels, 90.7%, 91.8% and 85.4% were found to be HCV antibodies positive by EIA formats A, C and D. The overall signal to noise ratio distribution for the HCV positive samples were found to be higher with Formats C and D which included a peptide (VIIIE) from the HCV core region than Format A which only employed peptides from the HCV nonstructural region as the solid phase antigen.

Except for one HBc antibody sample where the results is borderline positive (S/cutoff ratio~1.0) with the HCV EIA Format A, Format C incorporating peptides (IIH, V and VIIIE) from both the HCV structural (core) and nonstructural regions was the most sensitive. The significant improvement in sensitivity makes Format C an ideal candidate for a HCV antibody screening assay.

(43)

EXAMPLE 16

Comparison Of Test Results Using The Three Peptide Based HCV EIA Formats (A,C And D) On Low Risk Random Blood Donors

Representative 264 donor specimens obtained in a blood bank setting were tested by all three EIA formats.

The results are shown in Figures 13-1 to 13-6. The frequency distributions of the peptide based HCV-EIA signal to cutoff ratios suggested an initial reactive rate of 1.13%, 3.0% and 3.0% with formats A, C and D respectively. The negative samples have a relative low signal to cutoff ratio in all three assay formats(see Figures 13-1, 13-3, and 13-5). Upon repeat testing, a repeatably reactive rate of 1.13%, 1.9% and 1.9% were obtained for formats A, C and D respectively. Among the

corresponding EIA ratios (Table 9). Among the eleven marked specimens, most showed an increased level of GOT/GPT and were associated with frequent episodes of elevated GPT previously. All eleven specimens scored negative by the rDNA HCV C-100 based EIA. However, these same samples reacted strongly (with O.D. ≥ 1.5) in the peptide based HCV EIA Format C. Since peptide VIII(=VIIIE) was synthesized according to amino acid sequences selected from the conserved structural (core) protein region, its inclusion in the peptide based HCV EIA (such as format C) will be particularly suitable when testing specimens from geographically distinct regions where a higher chance of strain-to-strain variation among the HCV isolates may be encountered. (44)

It is to be understood that the above examples are illustrative of the present invention and are not meant to limit the scope thereof.

Table 8

Testing of Various Formats of HCV EIAs with Three Well-Characterized Seroconversion Panels

Panel	Donor #	Bleed Date	ALT (IU/L mu/mL)	AST (IU/L mu/mL)	EIA Ratio: rDNA HCV c-100	EIA Ratio: HCV EIA Format A (ns)	EIA Ratio: HCV EIA Format C (core+ns)	EIA Ratio: HCV EIA Format D (core)
Panel 1	021900	880809	40.0	NA	0.03	0.093	0.108	0.205
		880816	32.0	NA	0.04	-0.014	0.045	0.129
		880823	32.0	NA	0.06	-0.050	0.025	0.072
		880830*	180.0	121.0	0.04	-0.050	1.037*	1.096*
		880928	401.0	352.0	0.19	0.100	7.193	7.703
		881109*	NA	NA	6.57*	16.700*	10.185	7.281
		881122	NA	NA	6.57	16.671	9.770	9.321
Panel 2	012698	880815	39.0	NA	0.0	0.014	-0.058	-0.008
		880825	274.0	310.0	0.0	0.443	0.058	0.108
		880829	346.0	270.0	0.0	0.029	0.128	0.185
		880914	1175.0	722.7	6.5*	4.057	7.435*	5.984*
		881005	429.7	172.3	6.5	5.857	7.811	5.851
Panel 3	206300	880829	63.0	65.0	0.04	-0.043	0.115	0.181
		880901*	81.0	NA	0.04	0.043	1.607*	1.108*
		880908	183.0	174.0	0.02	-0.043	2.506	3.116
		880928*	563.0	555.0	6.57*	3.800	9.827	9.659
		881026	436.0	151.0	6.57	13.786	10.630	10.566

(47)

Table 9

HCV Positivity in Serum Specimens
Obtained from Japanese Dialysis Patients

Code No.	rDNA based HCA EIA OD Cutoff = 0.40	Peptide based HCV EIA Format A Cutoff = 0.205	Peptide based HCV EIA Format C Cutoff = 0.204	HBsAb	GOT/GPT Oct. 89	n: times during 1986-1988 when GPT ≥25 IU/L
24	0.058	-0.001	0.005		2/3	0
25	0.042	0.005	0.007		9/9	0
26	0.105	-0.001	-0.003		4/4	0
27	1.837	1.462	2.312	-	3/6	2
28	1.797	1.637	2.398	-	20/21	2
29*	0.011	0.001	1.603		7/4	0
30	0.994	0.374	2.213		11/9	0
31	1.823	0.425	0.874	-	27/16	4
32	0.770	0.372	0.500	+	17/7	9
33	1.712	2.101	2.234	-	28/32	29
34	0.002	-0.003	0.007		11/14	0
35*	0.026	0.161	2.229	+	14/23	23
36*	0.065	0.018	2.286		20/18	
37	0.021	0.000	0.011	+	16/11	1
38	2.347	1.917	2.182	+	26/23	6
39	0.008	-0.007	0.004		7/6	0
40	0.026	0.006	-0.002		10/8	0
41*	0.061	0.118	1.933	+	9/6	
42	2.481	2.144	2.211	-	13/19	2
43	0.008	-0.005	-0.005	+	11/7	
44	0.009	-0.004	-0.005		4/4	0
45	0.009	0.000	-0.003		7/2	0
46	2.177	1.990	2.121	-	16/12	8
47	0.023	0.003	0.015		7/3	0
48	0.025	-0.003	0.002	+	18/11	
49	0.025	-0.001	-0.006		9/5	0
50	0.026	0.024	-0.003		9/3	
51	0.018	-0.003	-0.007	+	11/5	
52*	0.011	-0.003	1.366	-	33/52	29
53	2.251	1.296	2.218		8/7	0
54	0.050	0.017	0.040		10/7	0
55	0.020	-0.007	0.017	+	14/8	
56	0.033	-0.004	0.000		9/3	0
57	1.396	0.718	2.121	-	17/11	1
58	0.045	0.013	-0.003		13/12	
59	0.014	0.068	0.056		10/7	0
60	0.009	0.014	0.056	+	15/0	10
61	2.087	2.214	2.235	+	12/9	
62	0.171	0.001	0.003		11/7	0
63	1.121	0.529	2.383	+	18/10	
64	0.113	0.066	0.002		4/3	0
65	0.032	0.003	-0.003	+	7/5	3
66	0.039	-0.001	-0.002	+	11/6	
67*	0.049	0.037	2.119		16/11	

Code No.	rDNA based HCA EIA OD Cutoff = 0.40	Peptide based HCV EIA Format A Cutoff = 0.205	Peptide based HCV EIA Format C Cutoff = 0.204	HBsAb	GOT/GPT Oct, 89	n: times during 1986-1988 when GPT ≥25 ~~IU/L~~ 10
68*	0.177	0.638	2.000	+	24/25	33
69	0.027	0.007	-0.007		6/3	0
70	0.031	-0.006	-0.001		16/9	0
71	0.781	0.473	2.151	+	13/8	14
72	0.110	0.002	0.059		13/8	0
73	0.043	-0.002	-0.007	-	2/3	0
74	0.014	0.001	-0.004		2/3	0
75	0.053	0.000	0.019	+	15/8	
76	0.060	0.015	0.018		14/7	0
77	0.011	0.001	-0.004		8/8	
78	0.042	0.092	0.023		3/0	0
79	0.537	0.219	1.742	+	11/7	
80	2.615	1.713	2.428	+	18/16	12
81	2.509	2.265	2.294		9/4	
82	0.019	0.000	0.120		11/5	0
83	0.511	1.928	2.229	-	19/11	5
84	0.020	0.016	0.095	-	12/9	
85	0.013	-0.003	0.116		10/7	0
86	0.003	-0.005	-0.006		19/5	
87	0.031	-0.009	0.009		10/6	0
88	0.039	0.019	0.004		6/2	0
89*	0.273	0.223	2.055	-	10/8	8
90	0.045	0.026	-0.002	-	7/3	3
91	0.018	0.003	-0.002		5/8	0
92	1.974	1.127	2.189	+	11/23	22
93	0.893	1.113	2.226	-	24/19	5
94*	0.267	0.353	2.029	-	18/12	1
95	0.026	-0.010	0.000	-	34/73	0
96*	0.021	0.002	1.599	-	13/30	27
97*	0.246	0.037	1.779		15/9	0
98	2.412	1.904	2.236	-	3/9	

WE CLAIM:

1. A peptide composition comprising a peptide with an amino acid sequence selected from the group consisting of:

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

(1X)

wherein X is -OH or $-NH_2$; and

(xi) analogues, segments, mixtures, combinations, conjugates and polymers thereof.

2. A peptide composition according to Claim 1 comprising a combination of Peptides I, II, III and V and having the amino acid sequence:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

wherein X is -OH or $-NH_2$ and analogues thereof.

3. A peptide composition according to Claim 1 comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

(i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;